SPECIFIC AIMS

Cancer is the second leading cause of death in the United States, accounting for nearly 25% of all deaths; in 2015, over 1.7 million new cases were diagnosed, with over 580,000 deaths. Many of these cancers involve the dysregulation of kinases, which play a central role in cellular signaling pathways. Mutations, translocations, or upregulation events can cause one or more kinases to become highly active and cease responding normally to regulatory signals. As a result, much of the effort in developing treatments for these diseases (and perhaps 30% of current drug development) has focused on shutting down aberrant kinases with targeted inhibitors.

Tyrosine kinase inhibitors (TKIs) in particular have proven themselves powerful therapeutics in the treatment of human cancers. The high selectivity of some TKIs such as imatinib—which potently inhibits just a small fraction of the human kinome to treat chronic myelogenous leukemia (CML)—is believed to be responsible for their effectiveness and low toxicity. Unfortunately, even when selective TKIs are available, the inexorable emergence of resistance mutations limits the duration over which the patient will derive therapeutic benefit, requiring a switch to second- and third-line selective TKIs—if they exist—as resistance develops. Ultimately, drug resistance is thought to be the reason for treatment failure in over 90% of patients with metastatic cancer.

The development of *new* selective kinase inhibitors remains incredibly challenging due to the fact that these inhibitors are almost universally targeted toward the ATP binding site shared by all kinases, but must bind with high affinity to only one (or a few) out of more than 500 human kinases to minimize unintended effects. While the discovery of imatinib was hailed as a breakthrough for its ability to selectively inhibit Abl over closely related kinases like Src, it came as a great surprise when the crystal structure of imatinib bound to Src was nearly identical to the Abl-bound structure. Recent evidence from experiments and simulation has suggested that a previously underappreciated contribution—the energetic cost of populating the inhibitor-bound conformation—plays a critical role in imatinib's selectivity. While this effect has only been examined in the well-studied case of Abl/Src binding to imatinib, it has the potential to be much more general. **We hypothesize that exploiting differences in the energetic cost of confining the kinase to the binding-competent conformation is a route to selectivity in targeted kinase inhibition.** Here, we ask how much conformational reorganization energy contributes to the selectivity and affinity of current noncovalent clinical kinase inhibitors to determine whether existing inhibitors (perhaps inadvertently) exploit differences in these reorganization energies to achieve selectivity, and whether this difference can be exploited to engineer new selective molecules.

We use a combined experimental and computational approach to decompose inhibitor binding affinity and selectivity into contributions from kinase reorganization and binding to individual kinase conformations:

Aim 1. Create an energetic atlas of the conformations accessible to human kinase catalytic domains. Using the Folding@home worldwide distributed computing platform, we will use massively parallel molecular simulations to map the conformational dynamics of kinase domains, generating an atlas of thermally accessible conformations and associated energetics using the Markov state model approach we originally developed to study conformational states transiently populated during protein folding. We will validate this map through the use of acrylodan labeling at locations predicted to be sensitive to ligand-induced conformational changes.

Aim 2. Quantify the contribution of kinase reorganization energy to inhibitor selectivity and affinity. We will use a novel automated platform to express a diverse panel of recombinant kinase domains and a newly developed fluorescence assay to directly measure the affinities of noncovalent FDA-approved kinase inhibitors to the entire panel. Combined with alchemical free energy calculations to individual kinase conformations, we will dissect the contribution of kinase reorganization energies and direct binding affinities using models that integrate experimental and computational data.

Aim 3. Identify opportunities to exploit differences in reorganization energies to achieve selectivity. We will validate our model by engineering mutations computationally identified to modulate inhibitor selectivities via manipulating differences in reorganization energies rather than the affinity for the inhibitor-bound conformation. In parallel, we will identify new opportunities to exploit differences in reorganization free energies between closely related kinases and between wild-type kinases and variants with clinically-identified oncogenic activating mutations.

This project will have a number of important implications for human health and our understanding of the biophysical determinants of selectivity. Structure-based drug design efforts will immediately benefit from a detailed understanding of the importance of reorganization energy and conformational energetics in determining affinity and selectivity. The release of an atlas of kinase conformations and energetics will provide new opportunities for the rational design of both ATP-competitive and allosteric kinase inhibitors. In addition, opportunities to exploit differences in reorganization energies of closely related kinases or wild-type and oncogenic forms of the kinase can present new paths to achieving higher efficacy without incurring additional off- or on-pathway toxicity.